Exhibit 20

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Randomized, double-blind, prospective study to compare topical 5-aminolaevulinic acid methylester with topical 5-aminolaevulinic acid photodynamic therapy for extensive scalp actinic keratosis

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Conflicts of interest
None declared.

Summary

Background 5-aminolaevlinic acid methylester (MAL) and 5-aminolaevulinic acid (ALA) photodynamic therapy (PDT) are both effective treatment options for actinic keratosis (AK). While MAL is significantly more expensive than ALA, no studies have directly compared their efficacy in the treatment of extensive scalp AK.

Objectives To compare the efficacy and adverse effects of MAL-PDT with ALA-PDT in the treatment of scalp AK.

Methods Sixteen male patients aged 59–87 years with extensive scalp AK were randomized into a double-blind, split-scalp prospective study. Two treatment fields were defined (right and left frontoparietal scalp) and treated 2 weeks apart. These fields were randomized to receive either MAL or ALA as first or second treatment. MAL cream was applied for 3 h; 20% ALA cream was applied for 5 h. A blinded observer assessed efficacy comparing AK counts before and 1 month after treatment. Pain was assessed using a visual analogue scale at 3, 6, 12 and 16 min.

Results Fifteen patients completed treatment to both fields. There was a mean reduction from baseline in AK counts with the use of ALA-PDT of 6·2 ± 1·9 compared with 5·6 ± 3·2 with MAL-PDT ($P = 0·588$). All patients experienced pain which was of greater intensity in the ALA-treated side at all time points: 3 min ($P = 0·151$), 6 min ($P = 0·085$), 12 min ($P = 0·012$) and 16 min ($P = 0·029$). Similarly, duration of discomfort post-procedure persisted for longer following treatment with ALA when compared with MAL-PDT ($P = 0·044$).

Conclusions This study demonstrates that both ALA-PDT and MAL-PDT result in a significant reduction in scalp AK. There is no significant difference in efficacy. However, ALA-PDT is more painful than MAL-PDT in the treatment of extensive scalp AK.

The safety and efficacy of both topical 5-aminolaevulinic acid (ALA) and 5-aminolaevlinic acid methylester (MAL) photodynamic therapy (PDT) have been demonstrated in the treatment of scalp actinic keratosis (AK).[1–3] MAL is currently approved in Europe for the treatment of AK and basal cell carcinoma (BCC) whereas ALA is unlicensed. MAL demonstrates improved lipophilicity when compared with ALA,[4] allowing for enhanced lesion penetration and also a greater specificity for neoplastic cells.[5] As a result it has been suggested that MAL-PDT may be more effective than ALA-PDT. In Ireland, MAL (Metvix®; Photocure ASA, Oslo, Norway) is significantly more expensive than ALA (Porphin®; Mandeville Medicines, Aylesbury, Bucks, U.K.).

The main adverse event with both treatments is pain, which is described by most patients. The severity of pain varies from a transient discomfort to severe pain.[6] Our aim was to compare both efficacy and adverse effects of MAL with ALA-PDT of scalp AK in a split-scalp randomized, controlled double-blind study.

Patients and methods

Patients

This study received local ethics committee approval and all patients gave informed consent to participate. Sixteen

caucasian male patients with bald scalps and extensive scalp AK were selected for PDT. The mean age of patients was 71·2 ± 8·78 (range 59–87) years. One patient, following treatment to one side of the scalp, requested that the same treatment be applied to the contralateral scalp. This patient was withdrawn from the study and his data was not included in the primary outcome analysis.

Treatment procedure

Prior to treatment, patients were assessed and equal treatment fields were measured on the right and left frontoparietal scalp. The number of palpable AKs was recorded. The AKs were classified according to the most severe type present: grade 1 (not easily seen, slightly palpable), grade 2 (well developed, easily palpable) or grade 3 (hyperkeratotic) AKs. Hyperkeratotic AKs were treated with white paraffin gel to remove any keratotic debris. Patients were randomized so that half would receive ALA and half MAL as their first split-scalp treatment. This allowed patients to act as their own controls, and thus reduce confounding factors. The second field was treated separately, 2 weeks later, with the other treatment modality. Care was taken to avoid any overlap between treatment fields, as evidenced by the absence of any local reaction extending beyond the treatment margins at fortnightly review.

A visible layer of 20% ALA (30 g 100 cm^{-2} Porphin®) or MAL (30 g 100 cm^{-2} Metvix®), was applied to either right or left frontal scalp for a 5-h or a 3-h period, respectively. The area was occluded with Tegaderm® (3M, Loughborough, U.K.) and covered with a gauze dressing. Five hours after application of ALA and 3 h after application of MAL, the excess was wiped off.

Fluorescence was graded on a scale of 1–3, using a Wood's light after 5 h for ALA and after 3 h for MAL; 1 was light/pale; 2, moderate; and 3, strong. Patients received 50 J cm^{-2} at 50 mW cm^{-2} using a Waldmann PDT lamp MSR 1200 (580–740 nm) to the treatment field. Treatment time was 16 min 40 s. Irradiance was measured using a calibrated hand-held meter (International light 1400A with Selo33/F/W/QND52 detector with spectral shaping and neutral density filters, calibrated by D. Taylor, Gloucester, U.K.). All patients were cooled with a fan and refrigerated cold-water spray during treatment.

Evaluation

Pain was assessed using a visual analogue scale (VAS) (1–100 mm) at 3, 6, 12 and 16 min. Patients moved a counter along a 100-mm scale from 'no pain' to 'worst pain ever'. The flip-side of the scale indicated the scores, 0 being no pain and 100 the worst pain ever as previously described.[7] Nurses recorded the numerical pain score and patients were not aware of this value. If treatment had to be discontinued because of pain, the timing of this was recorded. Adverse effects were documented.

Patients were assessed for erythema and erosions 14 days after their first and second treatment by one investigator (F.M.) and asked to indicate at what time following treatment were they no longer aware of discomfort in the treatment site and whether analgesia was required following treatment. Patients were further assessed for clinical response 1 month after their last treatment by a second investigator (P.C.), who recorded patient preference and side-effect profile. He categorized response to treatment as clear, improved or no response, and recorded the number of residual palpable AKs. Patients were asked if they had a preference for one treatment modality. Both patients and investigators remained blinded until study completion.

Data analysis

Baseline AK counts, reduction in AK counts and pain scores were normally distributed as confirmed using the Shapiro–Wilk test (Stata Corporation, College Station, TX, U.S.A.); therefore, differences between treatment groups were tested by the paired Student's t-test. Nonparametric two-sided Wilcoxon signed ranks tests were employed to assess differences in the AK count and duration of discomfort post-treatment. Statistical analyses were performed with JMP IN version 5.1 for Windows (SAS Institute, Cary, NC, U.S.A.). P-values < 0·05 were deemed statistically significant.

Results

Baseline characteristics

All patients had diffuse AKs; 31·3% (five patients) had predominantly grade 1 AKs, 56·3% (nine patients) had predominantly grade 2 AKs and 12·5% (two patients) had scattered grade 3 AKs on a grade 2 background. There was no difference in the baseline number of AKs in the MAL and ALA treatment fields (P = 0·159) (Table 1). Fluorescence was noted as grade 2 in seven treatment areas and grade 3 in 24 treatment areas. No patient had taken pretreatment analgesia on either the first or second treatment.

Efficacy

Fifteen patients received treatment to both sides of the scalp. The median number of AKs on the ALA-treated fields 1 month post-treatment was 1 [interquartile range (IQR), 0–2] compared with a median of 2 (IQR, 0–4) in MAL-treated fields (Table 1). This represented a mean reduction from baseline in AK counts with the use of ALA of 6·2 ± 1·9 compared with 5·6 ± 3·2 with MAL (P = 0·588). One month following the last treatment seven of 15 fields (46·7%) treated with MAL-PDT were clear compared with six of 15 fields (40%) treated with ALA-PDT. This represented a clearing of 71% of AKs treated with MAL-PDT compared with 87% treated with ALA-PDT. Treatment failed, with no clinical response, in one field, which had received MAL-PDT. Blinded dermatologist

Table 1 Assessment of split-scalp treatment efficacy and patient preference for ALA-PDT and MAL-PDT

	ALA-PDT (n = 15)	MAL-PDT (n = 15)	P-value
Efficacy, n (%)			
Clear	6 (40)	7 (46·7)	
Improved	9 (60)	7 (46·7)	
No response	0 (0)	1 (0·7)	
AK count baseline			
Mean ± SD (CI)	7·3 ± 1·6 (5·8–8·7)	8·8 ± 1·5 (7·0–10·6)	0·159
Median	8	8	
Range	2–11	2–16	
AK count 1 month post-treatment			
Mean ± SD (CI)	1·1 ± 1·2 (0·4–1·7)	2·7 ± 3·4 (0·9–4·6)	0·227
Median	1	2	
IQR	0–2	0–2	
Reduction in AK count from baseline			
Mean ± SD (CI)	6·2 ± 1·9 (5·2–7·3)	5·6 ± 3·2 (3·9–7·5)	0·588
Median	7	6	
Range	2–9	0–12	
Patient preference, n (%)*	2 (13·3)	10 (66·7)	

ALA, 5-aminolaevulinic acid; MAL, 5-aminolaevlinic acid methylester; PDT, photodynamic therapy; AK, actinic keratoses; SD, standard deviation; CI, 95% confidence intervals; IQR, interquartile range.
*Three patients expressed no preference.

assessment indicated a better clinical response with ALA in six patients (40%), MAL in one patient (6·7%) and no difference in the remaining eight patients (53·3%).

Pain scores

Pain scores were higher for ALA-PDT when compared with MAL-PDT during treatment of scalp AKs (Table 2). This held true at all time points: 3 min ($P = 0·151$), 6 min ($P = 0·085$), 12 min ($P = 0·012$) and 16 min ($P = 0·029$). The duration of discomfort following treatment was significantly longer following ALA-PDT [median, 480 min (IQR 330–600)] when compared with MAL-PDT [median, 120 min (IQR 0–495)], $P = 0·044$. Three patients required oral analgesia following ALA-PDT compared with one patient following MAL-PDT.

Adverse events

One patient reported burning pain localized to the half-scalp treated with ALA-PDT, induced by natural or artificial light exposure. This was persisting at the 1-month assessment. He was not on phototoxic therapy. There were no other adverse events recorded apart from mild erythema in all treated sites and superficial erosions in two patients from both treatment groups noted at the 2-week assessment.

Patient preference

When asked which treatment they would prefer to receive if further treatment was required, ten of 15 patients favoured MAL-PDT because it was associated with less pain. Three patients expressed no preference while two patients chose ALA-PDT on the grounds that they perceived it to be more effective than MAL-PDT.

Discussion

Chronically sun-damaged scalps are commonly seen in elderly bald males. In a fair-skinned Irish population, such scalps often demonstrate diffuse visible and palpable AKs on a background of actinically damaged skin, so-called field cancerization. Our study has shown that ALA-PDT and MAL-PDT are both effective in the treatment of such scalps. A similar reduction in the number of AKs and clearance rate was achieved with both treatments. All patients experienced pain during treatment, the intensity of which varied. We demonstrated that MAL-PDT proved less painful for patients both during and following MAL-PDT treatment when compared with ALA-PDT.

To date, there have been many open studies, which have demonstrated the efficacy of both ALA-PDT and MAL-PDT in the treatment of AKs.[8–10] MAL-PDT is also effective in both

Table 2 Comparison of pain scores during ALA-PDT and MAL-PDT and duration of discomfort post-treatment

	ALA-PDT	MAL-PDT	P-value
Pain score, mean ± SD (CI)			
3 min	38·3 ± 28·2 (22·7–53·9)	25·9 ± 22·1 (13·7–38·2)	0·151
6 min	33·2 ± 23·5 (20·3–46·3)	24·4 ± 16·2 (15·4–33·4)	0·085
12 min	38·6 ± 24·4 (25·1–52·1)	21·6 ± 15·1 (13·3–29·9)	0·012*
16 min	37·3 ± 25·6 (23·2–51·5)	22·3 ± 15·7 (13·6–30·9)	0·029*
Duration of discomfort in min, median (IQR)	480 (330–600)	120 (0–495)	0·044*

ALA, 5-aminolaevulinic acid; MAL, 5-aminolaevlinic acid methylester; PDT, photodynamic therapy; SD, standard deviation; CI, 95% confidence intervals; IQR, interquartile range.
*Results that are significant at 5% level.

the treatment and prevention of AKs in immunosuppressed populations.[11,12] Further studies have compared MAL-PDT treatment of AKs with other treatment modalities such as cryotherapy[13–15] and topical 5-fluorouracil,[16] demonstrating similar response rates and cosmetic outcomes. Those studies, which employed a randomized controlled intraindividual design, have a greater power to demonstrate differences between the modalities being compared.

To our knowledge, no study has directly compared the efficacy of ALA-PDT with MAL-PDT in the treatment of AKs, despite the significant cost difference between the two preparations. Eight weeks after a single treatment using ALA-PDT, Jeffes et al.[8] recorded total clearing of 91% of lesions on the face and scalp. Tarstedt et al.[10] assessed patients 3 months after their last treatment and documented a complete response rate of 93% for thin lesions but only 70% for thicker AKs after a single MAL-PDT treatment. Drawing comparisons between the findings of such studies is obviously limited by differing study methodologies, treatment regimens and lesion selection. It is also important not to compare clearance rates of individual lesions treated with clearance rates for a treatment field. In our study, for example, the total AK count in patients treated with ALA was reduced by 87% at 1 month. This corresponds directly to 40% of scalp sites treated with ALA achieving total clearance at 1 month, while appearing to present a more favourable response. Follow-up studies will be required to assess whether response rates are maintained over longer time periods.

A number of approaches have been employed to reduce the pain associated with PDT. Two prospective, randomized studies, using tetracaine gel (Ametop®; Smith and Nephew, Hull, U.K.) in the first and a eutetic mixture of lignocaine 2·5% and prilocaine 2·5% (EMLA® cream; Astra Pharmaceuticals, King's Langley, U.K.) in the second, concluded that topical anaesthesia conferred no beneficial pain relief during PDT treatment.[7,17] We standardized our treatment using a fan and cold-water spray during treatment. We identified treatment fields with comparable areas and lesion size in keeping with the work of Grapengiesser et al.[18] showing that site and size of the lesion and individual patient characteristics were the main determinants of PDT-associated pain. To counteract the possibility of patient anxiety and unfamiliarity with the proposed treatment influencing pain scores, half received MAL-PDT and half ALA-PDT as their first treatment. In practice there was no difference in scores relative to which treatment was first received.

The pattern of pain intensity was similar for both ALA-PDT and MAL-PDT, with both treatments inducing pain, which gradually intensified during the first minute of treatment, reaching a maximum prior to the first recorded pain score at 3 min, and easing immediately on discontinuing treatment. Less pain with MAL-PDT when compared with ALA-PDT had been documented previously in normal tape-stripped skin.[19] All fields treated in this study had evidence of chronic ultraviolet (UV) damage. The field changes in skin that has been chronically UV damaged includes thickening of the stratum corneum, which in theory should reduce the penetration of ALA and MAL into target lesions. This was in part counteracted by removal of keratotic debris from the AK surface. It has been postulated that ALA but not MAL is transported by γ-aminobutyric acid (GABA) receptors present in peripheral nerve endings, thereby causing more pain.[20] However, a recent study has demonstrated that there is no significant difference in the penetration depths of ALA and MAL in normal skin but that ALA is more effective at inducing protoporphyrin IX (PpIX) relative to an equivalent concentration of MAL applied to normal skin.[21] These findings have yet to be reproduced in diseased skin.

The exact level of PpIX required in tissue to generate a clinically significant photodynamic effect is unknown. There is no agreed standard application time for ALA. Work which measured levels of PpIX in plaques of psoriasis after application of ALA showed that levels peaked at 5 or 6 h, reaching 80–90% at 4 h.[22] While it is possible that the longer duration of application for ALA, compared with MAL, could in itself influence pain severity and efficacy, there is no evidence to support this. An ideal comparison of efficacy would also compare PpIX distribution within the epidermis prior to irradiation. Robinson et al.[23] highlighted the importance of diffuse distribution of PpIX in the epidermis compared with localized hot spots of PpIX in the stratum corneum as a key determinant of efficacy.

Our statistically lower pain scores for MAL-PDT are mirrored clinically in the patient preferences should further treatment be required, a majority selecting MAL-PDT on the basis of its pain profile. Our findings support those of Kasche et al.[24] who asked patients with scalp AKs receiving either MAL-PDT or ALA PDT to rate their pain. They stopped treatment in 54% of patients treated with ALA-PDT due to unbearable pain, in comparison with 14% of those receiving MAL-PDT. No patients in our study required treatment to be discontinued.

The persisting photoaggravated burning pain experienced by one of our patients in the area of scalp treated with ALA-PDT is not a documented side-effect, nor had we experienced other patients with the same problem. In theory, all PpIX should be metabolized to the photodynamically inactive haem within 48 h;[25] however, PpIX has been demonstrated in plaques of psoriasis for up to 14 days following a single ALA application.[26]

The results of this study allow for the application of data-based rationale in choosing a mode of delivering PDT to sun-damaged skin. The absence of a demonstrable difference in the efficacy of ALA-PDT and MAL-PDT treatment of scalp AKs places greater emphasis on the side-effect profile and patient acceptability of these treatments. Despite optimizing pain-relief techniques during PDT treatment, pain remains a major parameter in our experience, often dissuading patients from further treatment, when severe. The results of this study have encouraged us to employ MAL-PDT in the treatment of scalp AKs, while reserving ALA-PDT treatment for those scalps that fail to respond.

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